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Effect of different irrigant solutions on microhardness and smear layer removal of root canal dentin

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Abstract

Aim: To compare the effect of different irrigants on root dentin microhardness and smear layer removal.
Materials and methods: A total of 50 roots were equally divided into two halves to measure dentin microhardness and to evaluate the amount of smear layer. One hundred root halves were divided into five equal groups 20 sample each according to the final irrigants used: Group 1: 2.5% NaOCl, Group 2: 2.5% sodium hypochloride (NaOCl) followed by 7% malic acid (MA), Group 3: 2.5% NaOCl followed by 17% ethylenediamine tetraacetic acid (EDTA), Group 4: 2.5% NaOCl followed by mixture of tetracycline, acid and detergent (MTAD) and Group 5: saline. Ten root halves from each group were prepared to measure dentin microhardness at baseline measurement and after treatment to determine the change in microhardness, while the remains 10 root halves were prepared for scanning electron microscope to evaluate the amount of smear in the coronal, middle and apical thirds.
Results: Data were analyzed using one-way ANOVA and Student's *t*-test for microhardness and Kruskul–Wallis and Mann–Whitney for smear layer. Malic acid showed the greatest significant reduction in dentin microhardness ($P < 0.05$), followed by EDTA, MTAD, NaOCl and saline (control). EDTA, malic acid and MTAD efficiently removed smear layer, respectively, in the coronal and middle thirds of root canal. However, in the apical region, malic acid showed more efficient removal of the smear layer than the other irrigants.
Conclusion: Malic acid is the most efficient final irrigant solution after NaOCl irrigation throughout instrumentation.

Keywords: Microhardness; Smear layer; Irrigant solution; MTAD

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1. Introduction

Success in endodontic therapy depends on chemo-mechanical debridement of the root canal system through the use of instruments and effective irrigant solutions [1]. Mechanical instrumentation of the root canals produces a smear layer composed of organic and inorganic substances such as dentin particles, necrotic debris, and odontoblastic processes. The smear layer is an amorphous irregular thin layer that covers the prepared canal walls and occludes the orifices of the dentinal tubules. It also hinders the penetration of intracanal medications and sealers into the dentinal tubules. Removal of the smear layer improves the fluid tight seal of the root canal system [2]. Effective cleaning of the canal system requires the use of irrigation solutions during instrumentation and irrigation, which serve variety of purposes including antibacterial action, tissue dissolution, cleaning and chelating [3].

Although some authors suggest retaining the smear layer because it acts as a barrier against bacteria and other irritants, its total removal is preferred to improve the adaptation of the filling materials to the root canal dentin, reduce apical and coronal microleakage of the root canal filling materials and facilitate the diffusion of the irrigants and medications to the root canal system [4].

Sodium hypochlorite (NaOCl) with a concentration ranging between 1% and 5.25% is the most widely used irrigant in root canal treatment and considered as an effective antimicrobial agent and an excellent organic solvent for vital, necrotic, and fixed tissues [5]. However, it is highly irritating to periapical tissues, especially at high concentrations. Therefore it should be used at the lowest effective concentration and should not be forced beyond the apex [6]. However, its capacity to remove the smear layer from the root dentin appears to be limited [7].

Chelating agents decalcify the dentin by combining with the calcium ions of the tooth structure, unlike acids, which dissolve the inorganic structure of dentin by their low pH[8].

The decalcifying effect of chelating agents depends largely on application time, solution pH, and concentrations [9]. Ethylene diaminetetraacetic acid (EDTA) is generally accepted as the most effective chelating agent in endodontic therapy. It is used to enlarge root canals, remove the smear layer, and prepare the dentinal walls for a better adhesion of filling materials. The disodium salt of EDTA at 17% concentration and neutral pH is widely preferred for root canal treatment [10].

Malic acid is a mild organic acid used as acid conditioner for dentin and enamel etching in adhesive dentistry because it can decalcify and chemically adhere to hydroxyapatite [11]. This material was suggested to remove the smear layer efficiently throughout the root canal with different concentrations (5%, 7%, 10%, or 15%) of malic acid but when it is used at concentrations more than 7% cause damage to inter-tubular dentin [12].

MTAD can eliminate microbes (eradicate *Enterococcus faecalis*) that are resistant to conventional endodontic irrigants and dressings [13,14]. It is also an effective solution for the removal of the smear layer when used as a final rinse [15].

It was found that the irrigant solutions can affect the microhardness of radicular dentin that consequently affects the clinical performance of endodontically treated teeth [16]. Apart from advantages of irrigating solutions such as flushing out debris, disinfection, smear layer removal, and lubricating dentinal walls, canal irrigants may induce adverse changes in physical properties of dentin, including the microhardness [17]. Although a reduction in microhardness facilitates the instrumentation throughout the root canal, it may also weaken the root structure [18]. Microhardness determination can provide indirect evidence for losing or gaining any mineral substance in the dental hard tissues [19].

Therefore it is important to study the effect of different irrigant solutions, NaOCl, Malic acid, EDTA and MTAD on both the microhardness of root canal dentin and smear layer removal as well as clarifying the correlation between smear layer removal and microhardness.

2. Materials and methods

Fifty straight single-rooted lower premolars with relatively similar dimension and morphology, freshly extracted with closed apices were collected from adult patients. Each tooth was radiographed to confirm the presence of a single canal. Teeth with previous root caries, cracks, curved canals, endodontic treatment, internal resorption or calcification were excluded. The selected teeth were cleaned from soft and/or hard attached tissues, decontaminated by immersion in 5.25% sodium hypochlorite solution for 30 min and stored in sterile saline solution at room temperature all over the study [8].

The crowns of all specimens were cut transversally at the cemento-enamel junction (CEJ) with double-faced diamond disc at low speed, with water coolant, to obtain a 15 mm ± 0.5 mm root length. The fifty specimens were randomly divided into two parts 25 each. The first part was used to test the surface microhardness of root canal dentin and the other part

was used to examine smear layer by scanning electron microscope (SEM).

2.1. Part I: microhardness evaluation

2.1.1. Specimen preparation

A total of 25 roots were used in this part. The root canals were instrumented up to master apical file number 50 K-file.[1] Cleaning and shaping was performed by step back technique and recapitulation using distilled water as an intracanal irrigant during instrumentation.

For longitudinal sectioning of the root, longitudinal grooves were made on buccal and lingual external root surface. These grooves were made using double-faced diamond disc at low speed with care not to penetrate the root canals.

Root specimens were then splitted with a chisel[1] into two segments giving 50 halves. Each prepared root half was horizontally embedded in acrylic block. Each root half was labeled on the acrylic block for indentation by a known private number after embedding in acrylic blocks during acrylic setting.

2.1.2. Measurement of microhardness

Microhardness was measured for each sample at baseline and after application of different irrigating solutions. Baseline microhardness testing was carried out using Vickers Microhardness Tester,[1,2] with a Vickers diamond indenter and a 20× objective lens to obtain pretreatment record for each individual half. The microhardness measurements were taken at three different points for each sample; on the cervical, middle, and apical thirds. Mean baseline Vicker hardness number (VHN) was calculated for each specimen.

The indentation was made on the dentin surface approximately 0.5 mm from the root canal space. Each measurement was carried out using a 200-g load for 15 s, oriented perpendicularly to the root surface. The diagonal lengths of indentations were measured by built in scaled micrometer and measurements were converted into Vicker's numbers.

2.1.3. Evaluation of microhardness for the tested irrigants

For testing the microhardness of the dentin surface after irrigation solutions, the specimens were randomly divided into 5 groups ($n = 10$) according to the irrigant used.

Group 1: 10 specimens were immersed in 2.5% NaOCl.
Group 2: 10 specimens were immersed in 7% Malic acid.
Group 3: 10 specimens were immersed in 17% EDTA.
Group 4: 10 specimens were immersed in BioPure MTAD.
Group 5: 10 specimens were immersed in normal saline (control group).

In order to prevent the dilution of the irrigants before the experiment, excess fluid was removed from the canal surface with sterile paper points.[1]

The root specimen of each section was immersed in the tested irrigant solutions for 5 min in closed glass plates at 37 °C [18].

All experimental specimens were then flushed with 30 mL sterile saline [18]. Specimens were dried with sterile paper points. The microhardness was measured for canal dentine surface after irrigation in the same way for each sample as baseline measurement to record the post-treatment VHN.

The change in microhardness was calculated as the difference between baseline values and post-application values after immersion in the tested irrigants.

2.1.4. Statistical analysis

The data were collected and tabulated for statistical analysis using SPSS computer software.[3] A descriptive analysis was computed as means and standard deviation for each group. Inferential statistical analysis was done using one-way analysis of variance (ANOVA) to detect the difference between tested groups and Student *t*-test was used to observe the difference between each two groups.

2.2. Part II: smear layer evaluation

2.2.1. Specimen preparation

A total of 25 roots were used in this part. The root canals were randomly divided into equal 5 groups according to the final irrigation solutions. The root canals were enlarged up to master apical file number (50 k-file) using step back technique. During

[1] Dentsply, Switzerland.
[2] Model HVS-50,LaizhouHuayian Testing Instrument Co., Ltd. China.
[3] SPSS company, Chicago, USA.

instrumentation, the canals of 4 groups were recapitulated and irrigated with 5 mL of 2.5% NaOCl while the fifth group was irrigated with 5 ml of 0.9% normal saline (control group). After completed instrumentation final irrigation was done with one of the tested solutions. A 30-G needle, which penetrated to within 1−2 mm from the apex, was used for irrigation. No instrumentation was performed during the final irrigation with the test solutions. The irrigant solutions were used in each group as follows.

Group 1: root canals that were irrigated with 5 ml 2.5% NaOCl the canal received no further irrigation and dried with sterile paper points.
Group 2: the final irrigant was 5 ml 7% Malic acid without instrumentation then the canal was dried with sterile paper points.
Group 3: the final irrigant was 5 ml 17% EDTA without instrumentation then the canal dried with sterile paper points.
Group 4: the final irrigant was 5 ml BioPure MTAD. It was left for 5 min inside the canal without instrumentation as recommended by the manufacturer then the canal dried with sterile paper points.
Group 5: (control group) the root canals were initially irrigated with 5 ml normal saline, then the canal dried with sterile paper points. No final irrigant was used for this group.

After final irrigation, each root canal was flushed and copiously irrigated with 10 ml distilled water and dried with absorbent paper point and the canals orifice were sealed with a small cotton pellet to prevent contamination of the root canal space during sectioning procedures. Two longitudinal grooves were prepared on the palatal/lingual and buccal surfaces of each root using a diamond disc, avoiding penetration into root canals [18]. Each root was then split longitudinally into two halves using a mallet and a Stainless-Steel chisel giving 10 root halves for each group.

2.2.2. Scanning electron microscope preparation

The roots sections were coded and mounted on metallic stubs. Specimens were coated with 10×10^{-6} m of gold in gold sputtered[4] to render the surface electrically conductive and observed with SEM.[5] Various SEM photomicrographs were taken at a magnification of × 1000 at the coronal (10−11 mm to apex), middle (6−7 mm to apex) and apical (1−3 mm to apex) thirds of each specimen.

2.2.3. Data collection and analysis

The amount of smear layer remained on the surface of the root canal or in the dentinal tubules was scored according to the following criteria [20].

Score 1: no smear layer, orifice of dentinal tubules patent.
Score 2: less than 25% of canal area was covered by a thin smear layer and the dentinal tubule opening is visible.
Score 3: patchy distribution of smear layer up to 50% of canal wall area.
Score 4: the entire canal wall covered with a thin homogenous smear layer.
Score 5: a thick inhomogeneous smear layer covered the entire canal wall.

The data were tabulated for statistical analysis using SPSS computer software. A descriptive analysis was computed as frequency of each score for each tested group. Inferential statistical analysis was done using Kruskal−Wallis test to detect a significant difference between groups and Mann−Whitney for the difference between each two groups.

3. Results

3.1. Part I: microhardness results

All irrigating solutions decreased the microhardness of the canal dentin surface compared to the baseline measurements. Malic acid exhibited the highest reduction in canal dentin microhardness followed by EDTA, MTAD and NaOCl. Control group (saline) showed the least reduction in canal dentin microhardness. The baseline measurements microhardness of canal dentin of surface ranged from 51.84 + 11.04 VHN to 56.84 + 10.06 VHN with no significant difference between the tested groups.

The baseline microhardness value for each individual root sample was compared with its post-treatment value as shown in Fig. 1. The Malic acid showed the greatest change in microhardness between the baseline and post-treatment measurements, the change equal 14.15 followed by EDTA, MTAD, NaOCl and saline (control group) showed the least change in microhardness between the baseline and post-treatment measurements, the change equal 0.47.

[4] SPI Sputter Coater, USA.
[5] (JAOL,JSM-5200 LV, Japan) electron microscope unit, Tanta University.

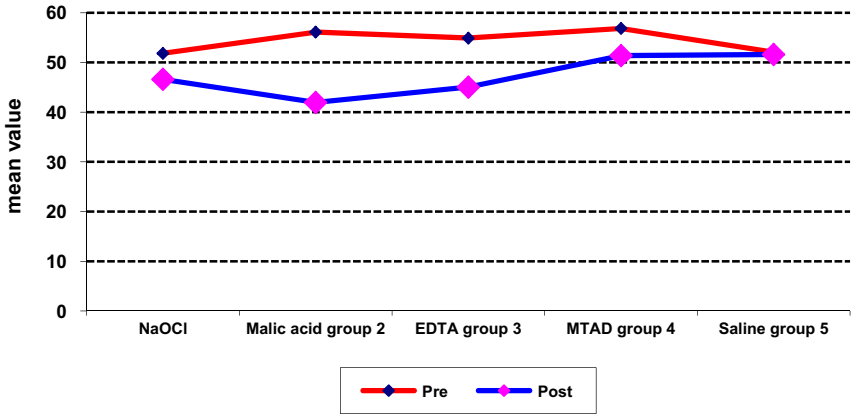

Fig. 1. Schematic diagram showing changes in microhardness values for the test groups. NaOCL, Malic acid, EDTA, MTAD and saline.

The change in microhardness was calculated and represented in Table 1.

Statistical analysis using one-way (ANOVA) showed that Malic acid, EDTA, MTAD and NaOCl have a significant reduction in root dentin microhardness ($P < 0.05$). Control group (saline) did not decrease the microhardness values significantly ($P > 0.05$). Malic acid induced the greatest reduction in root dentin microhardness ($P < 0.05$). There was no significant difference between MTAD and EDTA with respect to change in microhardness ($P > 0.05$), Table 1.

3.2. Part II: smear layer results

The smear layer scores are listed in Table 2 that exhibited the ability of malic acid, EDTA and MTAD to remove smear layer efficiently in the coronal and middle thirds of the root canal. However, in the apical region malic acid showed more efficient removal of the smear layer, while EDTA and MTAD showed heavy smear layer in the apical region. Specimens treated with NaOCl and saline showed thick smear layer in the three thirds of the root canals, Table 2 and Figs. 2 and 3.

Regarding NaOCl group, coronal and middle thirds showed score 3 (patchy distribution of smear layer up to 50% of canal root area), in two specimens and score 5 in eight specimens (thick inhomogeneous smear layer covers the entire canal wall). However, the apical third showed score 5 in all specimens, Table 2 and Fig. 2a. While for malic acid group, coronal and middle thirds showed score 1 (no smear layer, orifice of dentinal tubules are patent) in nine specimens and score 2 in one specimen (<25% of canal area was covered by a thin smear layer and the dentinal tubules opening is visible). However, the apical third showed score 1 in seven specimens and score 2 in three specimens, Table 2 and Fig. 3a.

When EDTA group was considered, coronal third showed score 1 in eight specimens and score 2 in two specimens, middle showed score 1 in seven specimens and score 2 in three specimens. However, the apical third showed score 3 in four specimens and score 5 in six specimens, Table 2 and Fig. 3b. For MTAD group, coronal third showed score 1 in six specimens and score 2 in four specimens, middle third showed score 1 in five specimens and score 2 in five specimens However, the apical third showed score 3 in two

Table 1
Reduction values and one-way ANOVA for microhardness of canal dentin surface for irrigant solutions.

Group	Amount of reduction	ANOVA	
		f Test	*p* Value
NaOCl	5.15	4.325	0.038[a]
Malic acid	14.15		
EDTA	9.86		
MTAD	5.47		
Saline	0.47		

[a] Significance, *p*-value < 0.05.

Table 2
Smear layer frequency scores within the coronal, middle and apical thirds of the root canals in test groups.

Group ($n = 10$)	Coronal					Middle					Apical				
	S1	S2	S3	S4	S5	S1	S2	S3	S4	S5	S1	S2	S3	S4	S5
NaOCl	0	0	2	0	8	0	0	2	0	8	0	0	0	0	10
Malic acid	9	1	0	0	0	9	1	0	0	0	7	3	0	0	0
EDTA	8	2	0	0	0	7	3	0	0	0	0	0	4	0	6
MTAD	6	4	0	0	0	5	5	0	0	0	0	0	2	0	8
Saline	0	0	0	0	10	0	0	0	0	10	0	0	0	0	10

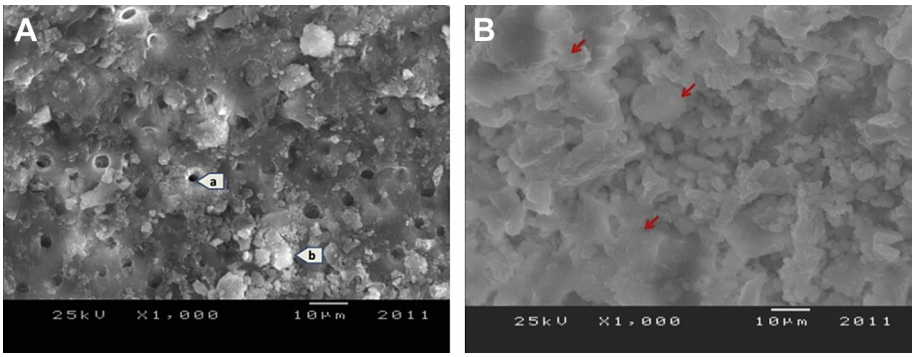

Fig. 2. SEM photomicrograph example of root canal wall treated with NaOCl (A) ×1000, with score 3 showing opening dentinal tubules (a) and patches of smear layers (b). Saline (B) ×1000, with score 5 showing thick smear layers (arrows).

specimens and score 5 in eight specimens, Table 2 and Fig. 3c. For saline coronal, middle and apical thirds showed score 5 in all specimens, Table 2 and Fig. 2b.

4. Discussion

Chemicals used during endodontic treatment may lead to alterations in root dentin and changes in its chemical and physical properties [21]. On the other hand, decrease in the microhardness can affect the adhesion and sealing ability of the sealers to the root dentin walls [17].

Irrigation with a tissue dissolving antimicrobial solution is a prerequisite for effective removal of the smear layer and remnant pulp debris which may in turn affect sealing ability of filling materials4. Therefore, this study was aimed to compare the effect of different irrigant solutions MTAD, Malic acid, EDTA and NaOCl on microhardness of the root canal dentin and their ability to remove smear layer.

4.1. Part1: microhardness evaluation

Microhardness of dentin may vary considerably between teeth, so in the present study the microhardness measurement was performed for each sample at baseline and after treatment with irrigation solutions to establish a reasonable evaluation for the effect of the irrigant solutions on the dentin surface. Post-treatment indentations were performed on each sample at same areas that were at symmetrical constant points of the baseline for both sides of the root canal to make evaluation of the tested irrigant [18].

Microhardness measurement was performed in three points at coronal, middle and apical third of the root canal dentin. Mean Vicker hardness number (VHN) was calculated for each specimen [22]. The microhardness of dentin depends on the tubular density which varies from an area to another on the root dentin surface. Therefore, the current study design followed Pashley et al. [23], who stated that the tubular density

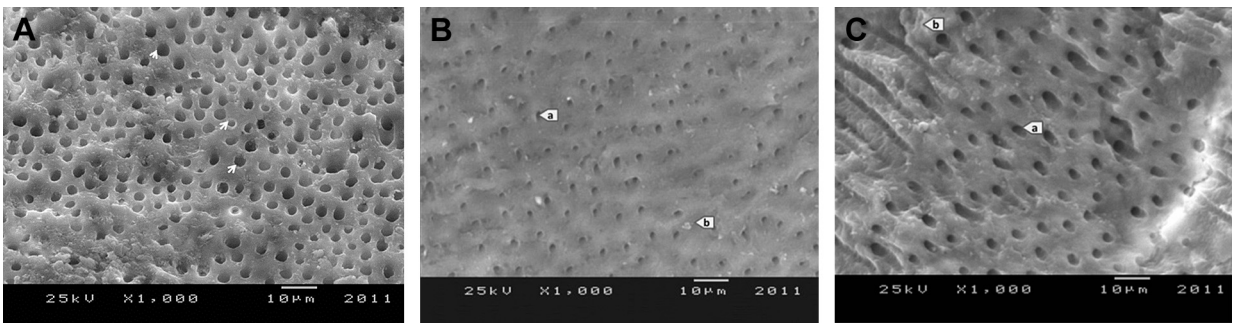

Fig. 3. SEM photomicrograph example of root canal wall treated with Malic acid (A) ×1000, with score 1 showing patent orifices of dentinal tubules and no smear layers particles in the examined field (arrows). EDTA (B) ×1000, with score 2 showing orifices of dentinal tubules (a) are patent and thin smear layer covered less than 25% of dentin canal surface (b). MTAD (C) ×1000, with score 2 showing orifices of dentinal tubules (a) are patent and thin smear layer covered less than 25% of dentin canal surface (b).

affects microhardness, as the tubular density increases dentin microhardness decreases.

Distilled water was used initially as an irrigant solution for microhardness specimens because it has no effect on dentin surface, thus not considered as a variable which might affect the results [24]. This followed by application of endodontic irrigation solutions on root canal dentin surface for 5 min in according to De Deus et al. [25], and Sayin et al. [26].

Selection of Vickers microhardness tester over Knoop hardness tester was due to the suitability and practicality of Vickers test for evaluating surface changes of deeper dental hard tissues. Knoop hardness tester is used for superficial dentin at 0.1 mm rather than for deep dentin [27].

A possible limitation of the current study is that the experiments were performed at room temperature and not body temperature. Additionally, the volume of the irrigant in a root canal clinically is small compared with the immersing root dentin in irrigating solutions. However, standardized circumstances for all study groups allowed for comparable results.

The present study revealed that all irrigation solutions decreased dentin microhardness with the exception of saline. This finding is in accordance with Saleh and Ettman [28], who evaluated the effect of NaOCl and EDTA on the microhardness of root canal dentin and reported that both solutions decreased the microhardness of root dentin but EDTA irrigation induced more reduction. Sayin et al. [26], also evaluated the effect of EDTA, EGTA, EDTAC and tetracycline HCL with and without subsequent NaOCl treatment on dentin microhardness and found that all tested solutions reduced dentin microhardness significantly. They concluded that significant alteration in dentin hardness after the irrigation treatment indicates potent direct effects of these chemical solutions on the components of dentin structure.

The chelating action of EDTA solution induces an adverse softening potential on the calcified components of dentin, and subsequently a reduction in the microhardness was expected. The organic-dissolving properties of NaOCl on the collagen component of dentin explain how the alternated irrigation with these solutions affects the hardness of dentin [29].

Sousa and Silva [30], Khedmat and Shokouhinejad [31]and DaSilva et al. [32], have shown that EDTA facilitates chelation of inorganic portion of dentin and NaOCl promotes dissolution of its organic portion. Regarding the depths under evaluation, the present study revealed similar results about reduction of dentin microhardness after irrigating with 17% EDTA followed by 2.5% NaOCl. This may be due to that 17% EDTA chleate mineral content (70%) of dentin; in other words EDTA demineralizes dentin and consequently makes it much weaker than normal.

Caltand Serper [33], studied the time-dependent effect of EDTA followed by NaOCl which can be the evidence for the dentin microhardness decrease. EDTA as a time-dependent solution after 5 min decreased dentin microhardness more than its 1-min application at a depth of 100 μm from the pulp−dentin interface. It is worth mentioning that EDTA in combination with NaOCl did not show a high dentinal penetration and consequently this combination may not be efficient in deep regions of dentinal tubules. Although NaOCl in combination with EDTA can decrease the dentin microhardness more than NaOCl alone [34].

The present study confirms the finding of Eldeniz et al. [35], who stated that EDTA and citric acid solutions had the strongest effect on reducing dentin microhardness compared with NaOCl and explained that EDTA acts efficiently in the reduction of dentin microhardness. The effect of EDTA was statistically similar to that of citric acid.

De Deus et al. [36], stated that although citric acid (as one of MTAD component) had the same concentration as that of the EDTA while the pHs were different. The more acid pH of a solution might favor the removal of calcium ions from dentin. Sousa and Silva [30], showed that 1% citric acid with pH 1.0 removed significantly more calcium ions from dentin than 1% citric acid with pH 7.4. The authors also stated that apple vinegar, acetic acid, and malic acid had a similar reducing effect on microhardness to each other and smaller than that of EDTA and citric acid. The lower concentration used for malic and acetic acids may be an explanation for such a result.

In the present study, malic acid induced the greatest reduction in root dentin microhardness, possibly because of its strong demineralizing effect owing to its high acidity and the ability to calcify root dentin, with most calcium and phosphorus extracted during its application compared with EDTA [37]. Ulusoy and Gorgul [18], stated that malic acid showed the greatest reduction in dentin microhardness followed by EDTA and MTAD.

The results of the present study differ from Ballal et al. [37−39], who stated that 7% malic acid reduced dentin microhardness as same as 17% EDTA. Spano et al. [40], used spectrophotometry atomic absorption to show that apple vinegar, 5% malic acid, and 5% acetic acid removed similar amounts of calcium ions

from the root canal but were less effective than 10% citric acid.

The current results also differ with that of Cruz-Filho et al. [21], who find that malic acid had a smaller reducing effect on microhardness than that of EDTA and citric acid. The lower concentration used for malic and acetic acids may be an explanation for such a result which is in accordance with Garcia-Godoy et al. [41], who stated that both MTAD and EDTA decrease the microhardness of root canal dentin because they made collapse of the dentin matrix structure.

The effect of MTAD on decreasing dentin microhardness may be attributed to its chelating components. The 3% doxycycline hyclate component of MTAD is an isomer of tetracycline. Tetracycline has a low pH and thus can act as a calcium chelator and cause root surface demineralization. Moreover, MTAD consists of 4.25% citric acid, which is capable of dissolving the mineral contents of dentin (67%) [42].

4.2. Part 2: smear layer evaluation

Presence of the smear layer prevents penetration of the sealers and endodontic materials into the dentinal tubules, which in turn causes apical microleakage. Removing the entire smear layer throughout the root canal is essential for the success of endodontic treatment. However, most irrigation materials come up short in removing the smear layer particularly in the apical third of the canals [43].

The entire canal length was utilized to test the efficacy of the solutions in all segments of the root including the apical third. Root canals in this investigation were prepared with the step back technique using hand instruments. This technique was reported to be an effective method to prepare root canals [44]. In addition, the use of the hand instruments creates a significant amount of smear layer [45].The canals were prepared apically up to size 50 K-file instruments to allow larger apical preparation according to Peters and Barbakow [46], who demonstrated that this size may improve mechanical removal of debris, microorganisms and allow adequate cleaning and penetration of the solution to the apical third of each root canal.

Injection the irrigant by syringe can control both the volume and depth of syringe penetration and the resulting flow of irrigant to the apical region of the canal system [47]. On this basis, all irrigations were done using 30-G needle as recommended by Plotino et al. [48]

Scanning electron microscopy has been used to determine the effectiveness of various irrigants to remove the smear layer. SEM allows an examination of morphologic details of the surfaces of prepared root canal [13]. However, the samples used in this study were single-rooted teeth with straight canal and the results may be limited only to such cases. Another limitation of this study was using hand instruments the use of rotary instruments may be more efficient in removing the smear layer.

The results of the present study showed that malic acid, EDTA and MTAD removed the smear layer efficiently in the middle and cervical thirds of the root canals, while only the malic acid succeeded to eliminate the smear layer from the apical third. This finding agrees with that of Mancini et al. [4], who found MTAD, citric acid and EDTA ineffective in removing the smear layer in the apical intraradicular dentin. The results of the present study are also in accordance with Ulusoy and Gorgul [18], who found that only malic acid succeeded to eliminate the smear layer from the apical third and no significant difference between NaOCl and saline in removing smear layer.

The results showed that NaOCl was an ineffective irrigant to remove the smear layer. These findings are similar to those observed in previous investigations Torabinejad et al. [13], Ulusoy and Gorgul [18], Mozayeni et al. [15], that showed these irrigants are not able to remove both organic and inorganic components of the smear layer.

The inefficiency of NaOCl to remove the smear layer from the dentinal walls may be due to its low physicochemical action that is limited to the organic particles. NaOCl coupled with EDTA can remove the inorganic debris formed in the instrumented root canals, mainly in the middle and cervical thirds [49].

The current study showed that both EDTA and malic acid were effective in removing of the smeat layer in the middle and coronal third of the root canal. However, 7% malic acid followed the 2.5% sodium hypochlorite had a better smear layer removal ability than 17% EDTA followed the 2.5% sodium hypochlorite in the apical third. These findings are in agreement with Torabinejad et al. [13] and Mancini et al. [4], that have reported EDTA to be effective in smear layer removal only in coronal and middle thirds but not in the apical third. The result of this study also agree with Ozdemir et al. [50], who stated that EDTA was not effective in complete removal of the smear layer in young and old root canal dentin.

Ballal et al. [38], reported that combined use of malic acid and NaOCl is more effective than EDTA in removing the smear layer in the apical third of the root canal. Final irrigation with 7% malic acid was reported

to improve the post obturation apical seal compared with 17% EDTA, which also supports the results of the present study.

The ability of malic acid to remove the smear layer in the apical third may be due to its highly acidity and its better demineralizing effect within a shorter period of time. EDTA was not able to remove smear layer effectively when compared with malic acid which may be attributed to the increased surface tension of 17% EDTA (0.0783 N/m) when compared with that of 7% malic acid (0.06345 N/m). Surface tension measurements of 17% EDTA and 7% malic acid were performed and found that surface tension of 17% EDTA is higher than that of 7% malic acid [2]. In addition, EDTA as a chelating agent is effective at a neutral pH and not dependent on a high hydrogen ion concentration to accomplish decalcification. The exchange of calcium from dentin by hydrogen results in a subsequent decrease in pH. Hence, the efficacy of EDTA decreases over time due to the decrease in pH [51].

Texeiraet al. [52], and Khademi et al. [53], showed that a neutral EDTA solution reduces the mineral and noncollagenous proteins (NCPs) component of dentin. Thus, EDTA not only removes calcium ions but also calcium bonded to NCPs. Because the content of NCPs decreases in the apical third of the root canal system, the degree of decalcification of EDTA in this part is low. The recommended amount of EDTA for the removal of smear layer varies from 3 to 20 mL per canal, but high-volume delivery is a hard task using a fine needle demanding a longer time and also fatigue to the operator.

There is no consensus on the optimum contact time which an irrigant solution to be kept in root canals for smear layer removal. Yamada et al. [54], suggested duration for 1 min with EDTA is sufficient. However, others advised a longer period of 15 min for optimal results. Paqueet al. [55], reported that dentin in the apical third of the root canal is sclerosed. Hence, EDTA may not have such a pronounced action on sclerosed dentin in the apical third, it requires an application time of not less than 15 min for optimal results.

The result of the current study are in accordance with Torabinejad et al. [13], who verified that 1% NaOCl preceding MTAD can dissolve the organic portion of smear layer that covers the dentinal tubules after instrumentation and this allows MTAD to dissolve the inorganic portion of the smear layer, penetrate into dentinal tubules, and decalcify them. Additionally, adding the detergent (Tween-80) can decrease the surface tension and increase the penetrating ability of MTAD [56].

5. Conclusion

Malic acid with an application time of 5 min induced the greatest reduction in root dentin microhardness as well as smear layer removal, followed by EDTA and MTAD.

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